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A NEW BI-FLAGELLATED PROTOZOOON OF MAN

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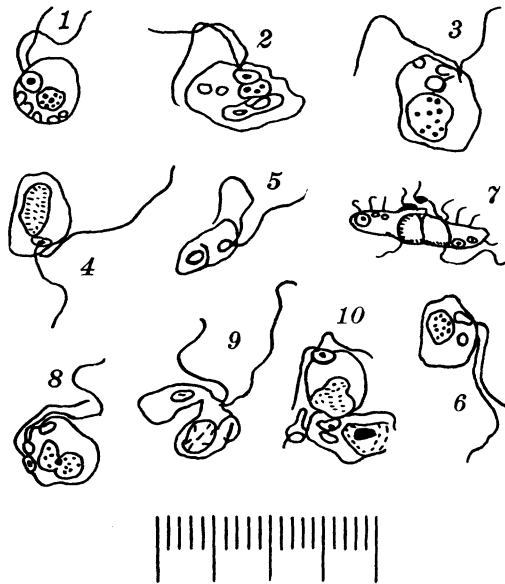
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In the study of postmortem cultures and direct smears from various organs of soldiers, we have found what appears to be a hitherto undescribed flagellate protozoon. This organism has been observed in three individuals, occurring respectively in the lung, the sphenoidal sinus, and the heart's blood. Its discovery, in the first place, was more or less accidental. It has been the custom here to supplement bacterial cultures with direct smears from the organs examined in order to study phagocytosis and other cellular phenomena. These smears were fixed in methyl alcohol, stained by Gram's method and counterstained in a weak, watery solution of eosin. Through an oversight a smear from the heart's blood of an influenza patient was left in the eosin solution for several hours; on examining the slide there were found flagellated organisms, and on examining the blood-agar plate culture of the heart's blood, similar structures were observed. Within a few days like protozoa were found in two new autopsies, both in direct smears and in the cultures from the sphenoidal sinus and the lung. Unfortunately the pressure of work during the epidemic of influenza did not permit us to investigate the flagellate closely at that time.

The original smears and slides made from the cultures were preserved after fixation in methyl alcohol; sublimate alcohol fixed slides were lost. Various stains were employed such as Giemsa's, Jenner's, eosin, methylene blue and eosin and iron hematoxylin. It was found that best results were obtained with weak watery solutions of eosin, staining over night. While the Giemsa preparations showed more clearly the internal structures, the flagella were generally so weakly stained that it was difficult to recognize them. It can be seen that our material was not fixed or stained by the best methods, and we are therefore unable to describe in detail the internal structures of the organisms. We realize that our studies are of necessity fragmentary and inconclusive, but we desire to record the findings in order to stimulate search for similar organisms.

MORPHOLOGY

The best preserved organisms were round or pear shaped and measured on an average of 6.5μ in their longest diameter, and 5μ in their greatest width. The extremes of measurements were 6 and 11μ for length, and 3 and 6μ for width. Near the more pointed end could be seen a small kinetonucleus, usually surrounded by a clear area. Sometimes two basal granules whence two flagella took their origin could be recognized near the kinetonucleus; it was a rule for them to have a distinct origin and to be well separated from one another at this



EXPLANATION OF FIGURES

Camera lucida drawings of various forms of the organism described. The small divisions on the scale are each one micron.

Fig. 1 to Fig. 6.—Typical organisms are shown possessing vacuoles, kinetonucleus, trophonucleus with chromatin granules or rods, and flagella.

Fig. 7.—Shows apparently division of trophonucleus. Adherent to the periphery of the protozoon are bacteria-like structures of unknown nature.

Fig. 8.—The trophonucleus is well divided.

Fig. 9.—Shows almost complete cell division.

Fig. 10.—Shows two adult protozoa adherent to one another.

point. These flagella were free, sometimes of equal and other times of unequal length, the shorter averaging 8μ , the longer 14μ . Toward the distal or more rounded end there was a large, trophonucleus, round or oval in shape and averaging 3.5μ in diameter. Coarse chromatin granules or rods could frequently be recognized; in several instances these appeared near the periphery of the nucleus in the form of a dis-

tinct ring. A small, well marked karyosome could often be seen within the nucleus. Within the cytoplasm, were generally several vacuoles; whether these be of contractile nature or food vacuoles we were unable to determine. The outline of the organisms was usually clear and distinct, but adherent to the periphery of several of the specimens were what appeared to be large bacteria.

Several examples were found which seemed to throw some light on reproduction. Thus, we have seen organisms with apparent division of their nuclear structures; others with a deep constriction in their body; and occasionally two fully formed protozoa were adherent to one another. It would seem then that these flagellates divide by binary fission. We also encountered small round bodies, averaging 4μ in diameter, showing the general internal structure, but no indication of flagella; these were looked upon as possible cystic stages.

Living forms were easily observed in cultures on rabbit's blood glycerine agar, on which we were able to cultivate the protozoa to the fourth generation at room temperature. These cultures were plentiful, always in association with various bacteria, chiefly streptococci and pneumococci. The organisms were actively motile, but the use of their flagella was not studied in sufficient detail. In cultures, but never in direct smears from the autopsy material, two other noteworthy structures were observed. One was like a large bacillus, the other like the head of a spermatozoon with a deeply staining granule. Both of these had a well stained yet delicate single flagellum, four to six times as long as their body. These structures might be connected with the genesis of the protozoon, but such a statement necessarily leads into speculation. Sure it is that none of the flagella of bacteria stained with dilute aqueous eosin; many of these bodies resembled the structures adherent to the wall of the protozoa.

DISCUSSION

The organisms described above occurred in every instance in cases of acute influenza. Careful analysis of the history of these patients and of the anatomical changes discovered at the autopsy brings out no additional information. The three patients seemed to have run a disease course exactly similar to that of other influenza patients of that period. As yet we have been unable to find the protozoa in the histologic sections from these cases and we have observed no microscopic tissue changes differing from those of other influenza patients. Klebs has described minute monads and attributed to them some rôle in the pathology of influenza. His observations have, however, not been confirmed. Since we have only found these protozoa in three bodies of 126 influenza necropsies studied in detail, and since the tissue of

these cases presented no unusual alterations, we would look upon the organisms as accidental invaders, possibly from the oral cavity. It is easily seen how such protozoa could make their way from the mouth cavity to the respiratory tract; their presence in the heart's blood can be explained by postmortem invasion from the lungs through the pulmonary veins.

Various flagellated protozoa have been described as occasionally inhabiting the oral cavity or the lungs. Thus, Fantham, Stephens and Theobald state "Prowazek speaks of a variety of *Trichomonas intestinalis* inhabiting the oral cavity. This was distinguished by a posterior process exceeding the length of the body fourfold, and by a somewhat unusual course of the undulating membrane. The food of this form, which was found in the whitish deposit present, especially in the cavities of carious teeth, consisted almost exclusively of micrococci. Schmidt and St. Artault named the Trichomonads found in pathological products (e. g., gangrene, putrid bronchitis, phthisis) of the lungs of man, as *Trichomonas pulmonalis*. Trichomonads have also been found by Wieting in lobular pneumonia in the lungs of pigs."

It is difficult to assign the protozoa which we have described to a definite place because of the insufficient data we possess. The fact that these organisms appear to constantly possess two flagella, places them in the family of Bodonidae, Bütschli, while the possession of a kinetonucleus would place them in the genus Prowazekia, Hartmann and Chagas, 1910. This, however, is only a tentative assignment.

SUMMARY

A small biflagellated protozoon was found in direct smears and in cultures from three postmortems of patients dead from acute influenza. They occurred, respectively, in the heart's blood, sphenoidal sinus and the lung, and apparently produced no tissue changes. The organisms were round or pear shaped, possessed two free flagella and a kinetonucleus. They were easily cultivated on rabbit's blood glycerin agar. We regard these organisms as accidental invaders, possibly belonging to the genus Prowazekia.

ARTICLES CITED

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